

The effect of varying levels of potassium on the frost resistance of birch seedlings

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TIIVISTELMÄ: ERI KALIUMPITOISUUKSIEN VAIKUTUS KOIVUN TAIMIEN PAKKASKESTÄVYYTEEN

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Seven hundred one-year-old *Betula pendula* Roth seedlings were given different concentrations of potassium fertiliser. Over the study period seedlings were subjected to artificial growing and dormant phases. Frost resistance of the seedlings was assessed by artificial freezing tests and electrical impedance measurements on stem cuttings. In general, high concentrations of potassium fertiliser reflected a low tolerance to frost. Pre-freezing impedance readings decreased with increasing potassium fertiliser dosages. Results from pre-freezing impedance measurements were found to be in broad agreement with the hypothesis that high impedance readings indicate a frost hardy tissue whereas low readings imply the opposite.

Eri kaliumlannoituksen pitoisuuksilla tutkittiin vaikutuksia seitsemänsadan yksi-vuotiaan koivun (*Betula pendula* Roth) taimen pakkaskestävyyteen. Tutkimuksen aikana taimet kasvoivat kasvihuoneessa ja lepokauden aikana ne olivat kylmiössä. Taimien pakkaskestävyys määritettiin keinotekoisessa pakastustestissä rungon leikeistä mitatuilla impedansseilla. Korkeat kaliumlannoitepitoisuudet ja alhainen pakkasensietokyky liittyivät yleensä toisiinsa. Jäädystä edeltävät impedanssit laskivat kaliumlannoitepitoisuuksien noustessa. Ennen pakastusta mitatut impedanssit tukivat hypoteesia, että korkeat impedanssilukemat ilmaisevat pakkaskestävää solukkoa. Matalat impedanssilukemat osoittavat päinvastaista.

Keywords: *Betula pendula*, electrical impedance, specific impedance difference. OCD 181.2+233.322+422.1+176.1 *Betula*

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1. Introduction

Frost damage to forest crops is an important practical problem in forest management. Because of their low metabolic rates during

the winter months plants have little opportunity to restore injured cells (Palta and Li 1978). Severe or sudden frosts produce struc-

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tural changes to cell membranes, essential proteins (e.g. APTase) which are bound to cell membranes, are damaged (Levitt 1972) and this in turn impairs the functioning of the plant's internal transport mechanism (Palta and Li 1978). The capacity to maintain high intracellular ion concentrations is also lost. The degree to which plants tolerate low temperatures depends on complex interactions between several factors (Tamm 1966), among which mineral nutrient concentrations in plant tissue may be important (Aronsson 1980). However, the way in which different nutrients influence frost hardiness is not fully understood.

Potassium plays a major role in the internal metabolism of plants and is required in cell production, protein synthesis (Evans and Wildes 1971), and the induction of growth in shoot tissue (Kramer et al. 1980). Potassium is highly mobile in the phloem and accumulates in young, actively growing areas of plants (Meyer et al. 1960). However, studies on the role of potassium in plant frost hardiness have produced conflicting results (e.g. Levitt 1956, Cristersson 1973, 1975).

Various methods have been used to evaluate injury caused by frosts. The most common of these are plant survival (e.g. Christersson 1971), relative conductivity (Carpenter et al. 1963), and electrical impedance (Glerum 1969, 1973, Pukacki 1982, Repo and Pelkonen 1986). The last method is based on the assumption that plant tissue is composed of resistive and capacitive components. Resistive components are affected by changes in the extra- and intracellular ion concentration gradients, which in undamaged cells are high. Capacitance is influenced by the status of the cell membranes. Other studies suggest that resistance is inversely proportional to capacitance (e.g. Tattar et al. 1974).

2. Materials and methods

2.1. Plant materials

Seven hundred one-year-old JR-1 type birch (*Betula pendula* Roth) paperpot seedlings

In a study by Rothschild (1946), impedance of cell membranes in healthy tissue was found to vary with frequency. At low frequencies the cell membrane impedance is measured while at high frequencies the cytoplasmic impedance is measured. In undamaged tissue the impedance at low frequency is high, and frost damage invariably results in a decrease in impedance (e.g. Wilner 1961, van den Driessche 1969, 1973). Whereas at high frequencies even after frost damage, impedance value remains about the same due to the electrical or ionic properties of the cytoplasm not being so severely altered as those of the cell membrane (Glerum and Krenciglowa 1970).

Previous studies have shown that impedance is dependent on several factors including temperature (e.g. Glerum and Krenciglowa 1970), tissue diameter (e.g. van den Driessche 1969), nutrient status of the tissue (e.g. Tattar et al. 1972, Pukacki 1982, developmental stage of the plant (e.g. Glerum 1973, 1980, Repo et al. 1984), water content (Glerum 1962), and the type of (Pukacki 1982) and distance between electrodes (Glerum and Zazula 1973).

The main objective of this report was to determine the effect of potassium on the frost resistance of birch seedlings. Additional objectives were to evaluate the use of impedance measurements for assessing the extent of frost damage and to determine whether potassium had any influence on the impedance reading of birch seedlings.

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grown at the Suonenjoki Commercial Nursery, Finland (62°40'N, 27°03'E 140 m asl) were used as the experimental material. The seedlings were of central and northern Finland

origin (62°N 27°E and 66°N 27°E respectively). Plants were taken at random from the nursery in January 1987. The seedlings were then transferred to the University of Joensuu and kept in open polythene bags in cold storage at +4°C.

During February, the seedlings were taken from cold storage and individually potted into 14.5 cm Ø pots, containing a 50:50 (v/v) mixture of unfertilised peat and fine sand. Prior to potting powdered dolomite limestone, (6.0 % Mg), had been thoroughly mixed with the substrate at 3 kg·m⁻³ peat. Finely ground micronutrient fertiliser (Puutarhan hivenoima, Fe 1.8%, B 0.4%, Cu 1.0%, Mn 2.0%, Zn 2.2%, Mo 0.004%, Se 0.0006%) was also incorporated into the soil at 0.7 l·m⁻³.

2.2 Experimental design

The seedlings were placed in a controlled greenhouse set to provide a temperature of +18°C and an 18 hour artificial and natural photoperiod. Supplementary lighting was provided by Airam H9LX 400 W mercury lamps. The seedlings were kept in these conditions from 1st March to 4th June 1987 inclusive. Because of external climatic conditions, the greenhouse temperature could not be held at a constant 18°C, and diurnal temperatures ranged from 25°C during the day to 13°C at night. The seedlings were watered regularly. Once budburst had occurred nitrogen (Kastelukkialpietari, 15.5% N) and phosphorus (ortho-phosphoric 85% acid, 31.6% P) were applied as separate solutions. Plants were fed 16 times over the growing phase of the experiment (approx. 1.25g fertiliser once per week).

Seedlings were divided into five treatment groups (140 seedlings/group) (Table 1). Potassium was given as K₂CO₃. To simplify procedures doses were made at the same times as those of nitrogen and phosphorus.

On the 23th March, 3 weeks after the first fertiliser application 20 seedlings from each treatment were randomly selected as sample material for the first freezing test. One further freezing test was carried out during the growing phase; 13 weeks after the first fertiliser application (Table 2).

Table 1. Total amount of nitrogen (N), phosphorus (P) and potassium (K) applied in g·m⁻² to the various treatment groups.

Treatment	Fertiliser applications g·m ⁻²		
	N	P	K
K0	20	20	—
K5	20	20	5
K10	20	20	10
K20	20	20	20
K40	20	20	40

Table 2. Times of freezing tests, and developmental phase of seedlings during the tests.

Test No	Date	Weeks after first fert. applic.	Developmental stage
1	23.3.87	3	Active
2	31.5.87	13	phase
3	26.6.87	17	Hardening
4	09.7.87	19	phase
5	21.8.87	25	

On the 4th June 1987 the remaining seedlings (approx. 100 seedlings/group) were removed from the greenhouse and subjected to cold temperature treatment at the University's botanical gardens. Due to lack of available unit space seedlings receiving different potassium treatments were placed in separate cold units. A 6 hour photoperiod was provided by fluorescent tubes (model Airam L65/80W 1×C "Daylight de luxe"). The internal temperature of the units was initially 5°C, but this was later reduced (13th June) to 3°C to hasten the onset of dormancy. No fertilisers were applied after the seedlings were placed in the cold units, but they were watered as necessary. Air circulation within the units was achieved by the use of electric fans which minimised the chance of formation of temperature gradients. Internal temperature was monitored by thermograph recorders. Daily minimum, mean and maximum temperatures were +2°C, +3°C and +6°C respectively.

Freezing tests were undertaken at various times over the cold temperature treatment period (Table 2).

2.3 Freezing procedure

Freezing tests carried out in a Lauda Compact Thermostate RKP20 freezing unit. Two stem cuttings each of 7 cm length were taken from the base of each sample seedling. Cuttings were placed in perspex testtubes, 8 mm \varnothing , and suspended in racks in the freezing unit. The internal temperature of the unit was decreased in steps to various preprogrammed levels (Fig. 1). At each assessment level 8 cuttings per treatment group were removed and left at room temperature for 30 minutes. Basic procedures were the same for all freezing tests; slight modifications in methodology were required in tests carried out during the dormant phase i.e tests 3,4 and 5.

2.4 Impedance assessments

The pre-freezing absolute impedance of each cutting was measured using a Finnish prototype HK1 device. Steel needle electrodes, 0.7 mm diameter, 7 mm long and 10.5 mm apart, were pushed through the cutting and the reading taken. A low frequency setting ($f=1\text{kHz}$) was used. Diameter of each cutting was measured to an accuracy of 0.1 mm. Post-freezing absolute impedance was measured on each cutting for the various assessment levels.

Impedance for these tests was standardised by calculating specific impedance difference from the equation (Repo and Pelkonen 1986)

$$\Delta z = (Z_2 - Z_1) A/l$$

where

- Δz = specific impedance difference Ωm
- Z_2 = post-freezing absolute impedance Ω
- Z_1 = pre-freezing absolute impedance Ω
- A = cross-sectional area of cutting m^2
- l = distance between electrodes m

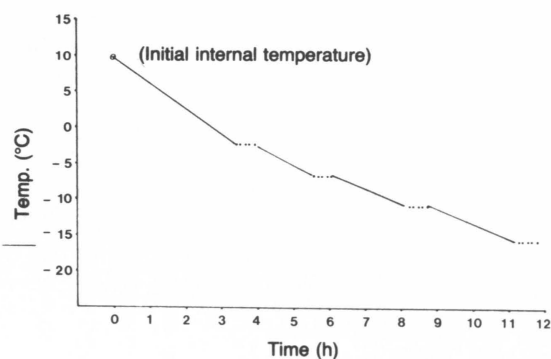


Figure 1. Example of preprogrammed temperature treatment within the freezing unit. Cuttings were held at assessment temperatures (-3°C , -6°C , -15°C in this case) for 30 minutes (...). 8 sample cuttings were then removed for impedance measurements. Rate of decrease in temperature to 0°C $5^\circ\text{C}\cdot\text{h}^{-1}$, and below 0°C decrease in temperature to 0°C was $5^\circ\text{C}\cdot\text{h}^{-1}$, and below 0°C was $2^\circ\text{C}\cdot\text{h}^{-1}$.

Repo and Pelkonen (1986) suggested that values of Δz below $0 \Omega\text{m}$ indicated the occurrence of frost damage. Furthermore they used the temperature corresponding to $\Delta z = -10 \Omega\text{m}$ as the indicator of frost resistance. For the purpose of this report a value of $\Delta z = -4 \Omega\text{m}$ was used as the critical assessment figure as this was the point which all treatments passed in all assessments. The corresponding temperature is indicated as $LT_{-4} \Omega\text{m}$.

2.5 Analysis of foliage nutrients

Foliage from the sample seedlings of each treatment was taken and fresh weights measured. All foliage samples were dried in an oven at a constant 60°C for 48 hours and dry weights then measured. Samples from tests 2 (leaves only) and 4 (leaves and stems) were analysed for concentrations of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg). All analyses were carried out at Viljavuuspalvelu (soil testing laboratory), Helsinki using the methods described by Halonen *et al.* (1983).

3. Results

3.1 Foliar nutrient concentrations

Foliar concentrations of potassium increased with increasing total amounts of applied potassium fertiliser at both sampling dates (Fig. 2). However, decreases in foliar concentrations of phosphorus, calcium and magnesium with increasing applied potassium fertiliser level were seen at both sampling dates. Nitrogen concentrations showed no significant differences between treatment groups at either sampling time. Foliar concentrations of all nutrients except nitrogen increased between sampling times in all treatments (Tables 3a-b).

3.2 Impedance measurements

Pre-freezing absolute impedance (20°C) decreased with increased level of potassium. In tests 2-4 impedance increased slightly from the pre-freezing measurement and then decreased with decreasing treatment temperature in all treatment groups (Fig. 3), except at the highest potassium level (K40) where impedance decreased constantly with decreasing temperature. Similar trends were observed in test 1 and 5 but in these cases, the K5 treatment produced erratic results and trends were not clearly seen.

Although changes in impedance with treat-

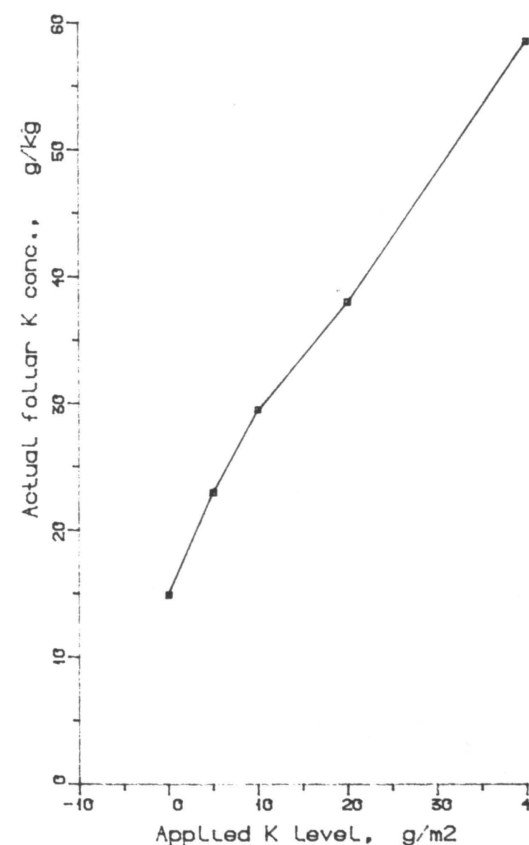


Figure 2. Actual foliar K concentration ($\text{g}\cdot\text{kg}^{-1}$) as a function of applied K treatment level (m^{-2}) for test 4.

Table 3a. Concentrations of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) for the treatment groups during assessment date 2 (31.5.87 leaves only).

	Foliar analysis $\text{g}\cdot\text{kg}^{-1}$				
	N	P	K	Ca	Mg
K0	35.9	8.6	15.2	20.5	6.7
K5	34.1	6.5	22.2	15.6	5.0
K10	34.0	5.3	24.0	13.2	4.2
K20	34.6	5.3	32.8	12.6	3.8
K40	34.7	4.8	45.6	9.2	2.9

Table 3b. Concentrations of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) for the treatment groups during assessment date 4 (9.7.87 leaves only).

	Foliar analysis $\text{g}\cdot\text{kg}^{-1}$				
	N	P	K	Ca	Mg
K0	31.5	9.0	14.9	26.3	7.3
K5	30.3	7.0	23.1	20.0	5.5
K10	32.5	6.9	29.5	18.8	5.0
K20	33.9	7.1	38.0	15.0	3.8
K40	34.1	6.7	58.6	12.6	3.5

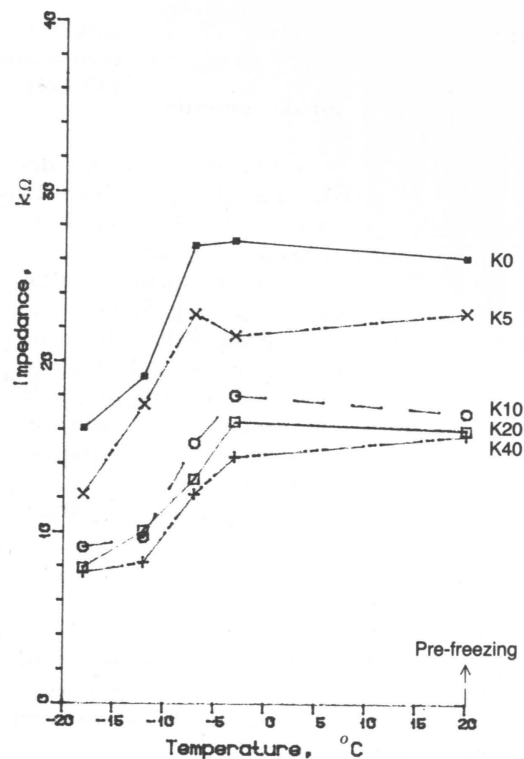


Figure 3. Absolute impedance as a function of exposure temperature for different fertiliser treatments in test 4 (Table 2).

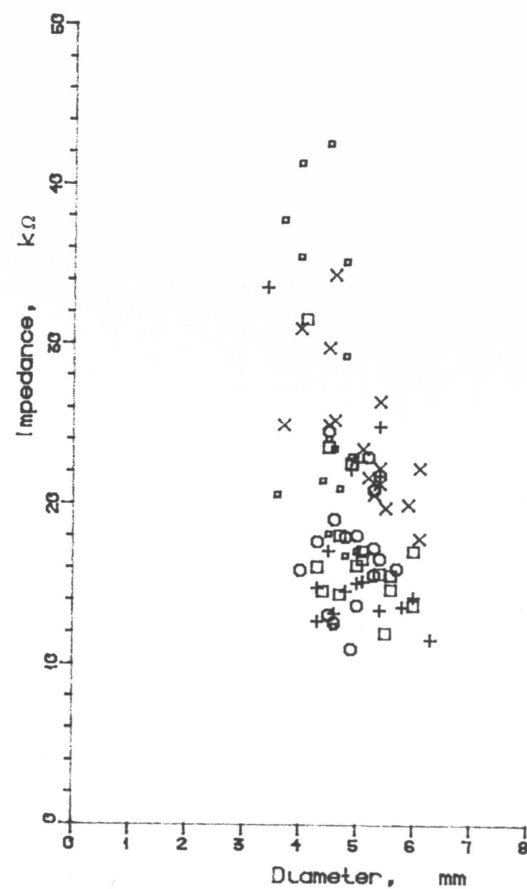


Figure 4. Pre-freezing impedance readings and diameter of sample cuttings for each treatment. Seedlings had been kept 3 weeks in 3°C. Test 4 (Table 2).
Key □=K0; X=K5; O=K10; □=K20; +=K40

ment temperature were similar in all treatment groups, the final post-freezing impedance value of the control treatment (K0) remained consistently higher than the pre-freezing impedance value of the K40 treatment.

Overall, data from tests 3–5 showed that cutting diameter and impedance were independent of each other (Fig. 4).

Results from specific impedance difference showed that seedlings in the K0 and K5 treatments had lower critical temperatures than seedlings given higher concentrations (Fig. 5a-c). The critical temperature to which treatments were able to tolerate freezing was shown to reach a maximum during test 5

(Fig. 6). The control treatment (K0) showed the greatest increase in frost tolerance withstanding freezing to -18°C . The final tolerance series being $\text{K0} > \text{K5} > \text{K10} > \text{K20} > \text{K40}$.

There appeared to be a negative curvilinear relationship between foliar potassium concentration and post-freezing impedance (Fig. 7a-b), with the curve becoming asymptotic at high levels of potassium. A decrease in the degree of curvature was observed between sampling times.

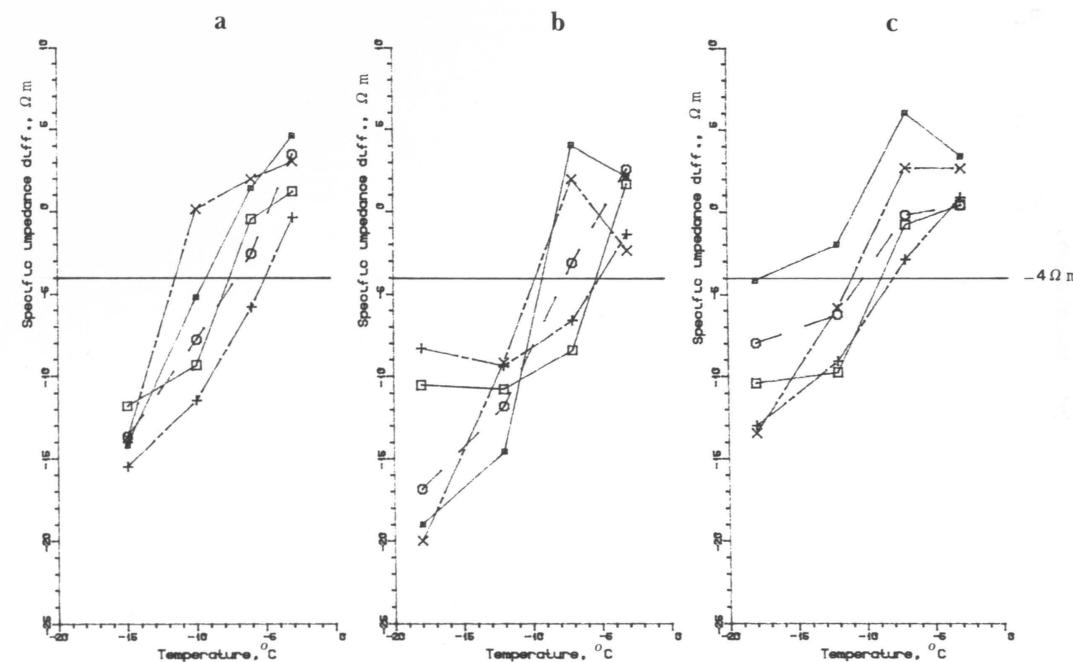


Figure 5. Assessment of frost resistance for the various treatment groups during the hardening phase. Specific impedance difference (Δz) as a function of frost treatment temperature. Temperature corresponding to a value of $\Delta z = -4 \Omega\text{m}$ taken as the indicator of frost resistance. (a) Test 3 (b) Test 4 (c) Test 5 (Table 2).
Key □=K0; X=K5; O=K10; □=K20; +=K40

4. Discussion

Treatments given low fertiliser dosages of potassium appeared, overall, to have a greater tolerance to freezing (Fig. 6). Results showed that between treatments, differences between critical temperatures became greater only after cold storage (Fig. 6). This implies that actively growing seedlings can be damaged within a narrow temperature range and that at this stage the nutrient status of the plant has little influence on frost resistance. However, during hardening the critical temperature range at which damage occurs increases. Thus, one interpretation of the findings is that hardening had started in treatments K0 and K5 at a time when plants in other treatments were still actively growing.

Low foliar concentrations of potassium have been thought to be one factor contribut-

ing to the likelihood of increased frost damage. However, results obtained by Christerson (1973) and Aronsson (1980) have shown that plants given relatively high doses of potassium still suffered frost damage. Ingestad (1974) suggested that maximum plant growth is only achieved if the plant has a balanced supply of nutrients. It may also be true that a plant with a well balanced nutrient supply maybe able to withstand adverse climatical stress better than a plant receiving imbalanced nutrients. Although the results from foliar analysis showed that no treatment had the exact optimum nutrient proportions for birch proposed by Ingestad (1962), foliar concentrations of potassium for the K0 treatment ($15 \text{ g} \cdot \text{kg}^{-1} \text{ K}$, Tables 3a-b) came closest to the ideal, relative to nitrogen con-

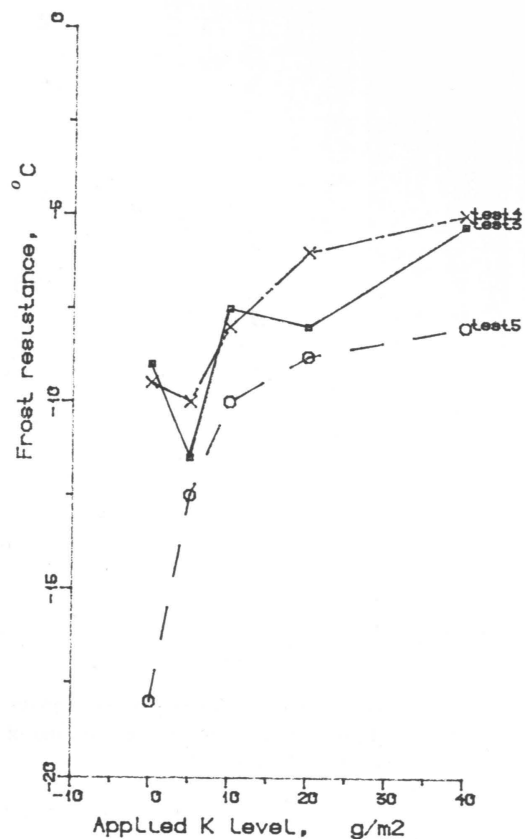


Figure 6. Frost resistance ($LT_{-4} \Omega m$) as a function of treatment temperature, during tests 3, 4 and 5. Assessment based on specific impedance difference curves (Figs. 4).

centration (taken as 100 %), being 45 % and 47 % in tests 2 and 4 respectively. During test 4 K5 had the greatest tolerance to low temperatures (Fig. 5b); however, difference in critical temperatures between K5 and K0 treatments at this stage was quite minimal. Whereas the frost tolerance of the K0 treatment in test 5 surpassed all other treatments. Interpretation would have been further helped by foliar analysis at the fifth sample date. Although an increase in potassium concentration has the overall effect of reducing frost tolerance, there appears to be a level ($20 \text{ g} \cdot \text{m}^{-2} \text{ K}$) where a further increase in potassium concentration produces only a slight decrease in tolerance (Fig. 6).

Pre-freezing absolute impedance of birch seedlings decreased with increasing levels of

potassium fertiliser (Fig. 3). These results reflect the high conductive nature of K^+ ions, this is due to an increase of electrolytes in solution which produces a reduction in the resistive components of tissues and thus a decrease in impedance. Shortle et al. (1976) observed that differences in the vigour of red maple (*Acer rubrum* L.) stems were reflected by changes in electrical resistance. They suggested that greater concentrations of mobile ions are present within the cambial zone of healthy stems than in stressed trees. Similar results were obtained by Tattar et al. (1972), Working with shoots of sugar maple (*A. saccharum*). They found a correlation between resistive impedance ($f=100 \text{ Hz}$) and concentration of potassium and calcium ions within the shoot. In both studies resistance/impedance was found to be inversely proportional to the concentration of mobile ions in tissues.

Pukacki (1982) observed a slight decrease in the impedance of *Magnolia* shoots as potassium and sodium concentrations increased. The influence of potassium was greater when the shoots had been frozen. Although it is known that the leakage of K^+ ions from cells increases after injury (e.g. Pukacki and Pukacka 1987), post freezing impedances recorded in this study (Fig. 7a-b) do not necessarily indicate that greater damage is incurred in tissues with high potassium concentrations. Rather the results may reflect both injury caused by freezing and initial applied concentration of potassium ions.

The use of electrical impedance techniques in physiological studies has increased in recent years, although as early as 1922 Osterhout had first observed that electrical impedance was a reliable indicator of plant health. Glerum (1980) suggests that high impedance readings indicate a frost hardy tissue whereas a low reading implies the opposite. Results from pre-freezing impedance measurements were in broad agreement with this hypothesis, though they were not always consistent (Figs. 3 and 4). Pelkonen and Glerum (1985) found that pre-freezing impedance was a poor indicator of post-freezing survival of poplar clones. Svejda (1970), working with rose tissues observed no significant differences in impedance readings between hardy and non-hardy tissue. Such differences in results suggest that further investigations are required.

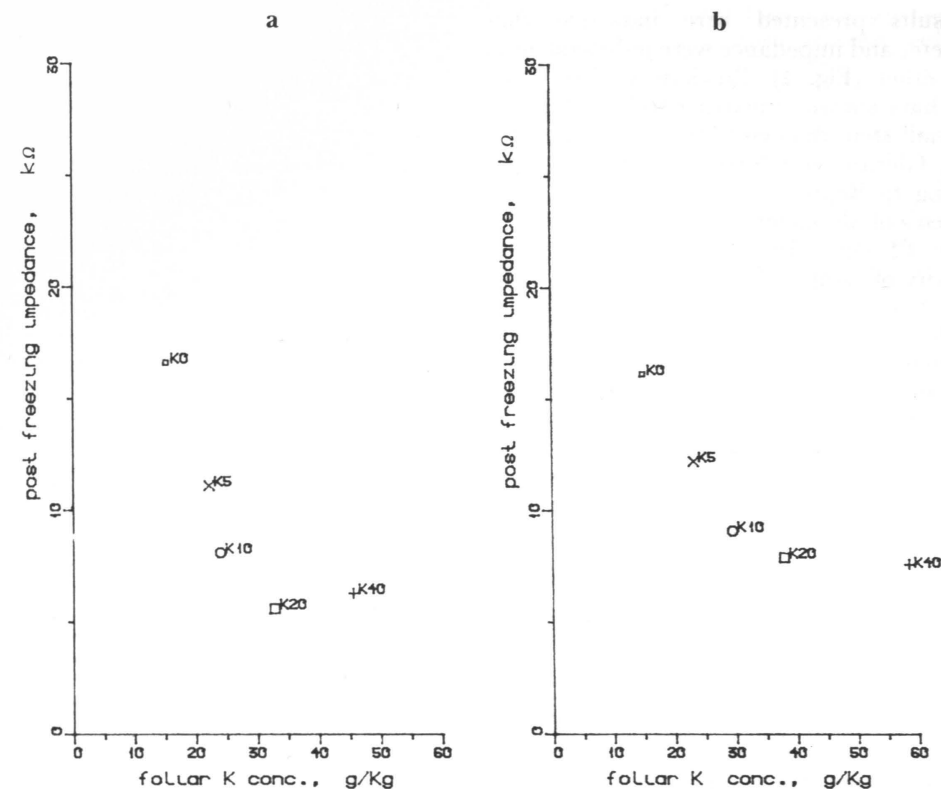


Figure 7. Impedance after freezing to -18°C as a function of foliar potassium concentration. (a) Test 2 and (b) Test 4 (Table 2).

Specific impedance difference calculations produced a more accurate account of frost resistance. A sharp increase in the impedance difference is observed as the treatment temperature nears the point at which frost damage occurs. According to Repo and Lappi (1989) the treatment temperature corresponding to an intermediate value of Δz can thus be used as a single indicator of frost resistance. However, the point taken to evaluate the critical temperature is quite arbitrary. Repo and Pelkonen (1986) found that the temperature corresponding to a value of $\Delta z = -10 \Omega m$ was approximate to the LT_{10} value for Scots pine during the dehardening phase. In this present study $\Delta z = 4 \Omega m$ was found to be the best base value for comparisons between assessment dates, as it was the only figure which all treatments passed. According to Repo and Lappi (1989) the standard error of the impe-

dance modulus is mainly due to the contact between electrode and tissue. The contact area may differ between measurements if the electrode position doesn't coincide with the initial diameter. Repo (Tapani Repo, Finnish Forest Research Institute, Suonenjoki Research Station, unpublished) found that the specific impedance value may also vary within the shoot. Test procedures, throughout this study, suggest that errors arise when electrodes are changed even though electrode type remains constant. This is thought to be the reason for discrepancies (tests 1 and 5) in impedance readings obtained in this present study. However, instability of the readings may also be due to the diffusion of air into the contact area (Repo and Lappi 1989), resulting from a lack of electrolytic fluid around the electrodes. Therefore, this too may have had an influence on impedance readings during this study.

Results presented here indicated that diameter and impedance were independent of each other (Fig. 4). Previous studies, however, have shown impedance to be influenced by small stem diameters (van den Driessche 1969, Glerum and Krenciglowa 1979). According to Repo (1988) impedance is independent of diameter when cutting area is above 12 mm². In this present study the majority of samples had a cutting area \geq 12 mm². Evert (1973) found that with samples which had large diameters, the majority of the stem consisted of wood and thus, the normalised impedance magnitude was independent of diameter.

Impedance measurements have been found to be a reliable tool for defining the critical temperature range during the dehardening, active and hardening phases of pine (*Pinus*

sylvestris) and spruce (*Picea abies*) seedlings (Repo and Pelkonen 1986, Rikala and Repo 1987). Impedance techniques are a potentially rapid and accurate way of determining the frost hardiness of plants, though readings should be measured under uniform conditions to avoid ambiguous errors. Furthermore, results from specific impedance difference (Fig. 5a-c) were more accurate in defining the critical temperature of frost resistance than was absolute impedance.

No definite conclusion can be drawn from this present study on the usage of potassium fertiliser for inducing a certain level of frost resistance in forest seedlings. However, it seems from the results that low levels (0–5 g·m⁻² K which is equivalent to 0–50 kg·ha⁻¹ K) may be sufficient for achieving a high frost tolerance in birch seedlings.

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